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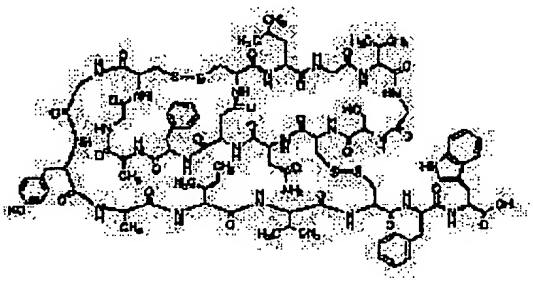
(54) PEPTIDE COMPOUND, ITS PRODUCTION AND ANTI-HUMAN IMMUNODEFICIENCY VIRAL AGENT

(57)Abstract:
PURPOSE: To obtain a low-toxic new peptide compound excellent in anti-human immunodeficiency viral activity, thus useful for AIDS treatment, etc., as an anti-human immunodeficiency viral agent, by culturing peptide compound- productive bacteria belonging to Streptomyces.



CONSTITUTION: This new peptide compound expressed by the formula is obtained from a cultured product obtained by culturing peptide compound- producing bacteria belonging to Streptomyces [e.g. Actinomycetes SKH-2344 strain (FERM P-14239)]. This compound, which is excellent in anti-HIV activity and has low toxicity, can be taken in large quantities for a long period of time, thus being highly useful as a new AIDS medicine. Specifically, this compound is obtained by the following process: Streptomyces SKH-2344 strain is cultured in a medium at 25° C for 5 days followed by centrifugation and then conducting a microbial collection, the resultant microbes are bacteriolyzed in methanol followed by making an extraction, and the extract thus obtained is concentrated to dryness, and the resultant extract is subjected to column chromatography and purified.

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* NOTICES *

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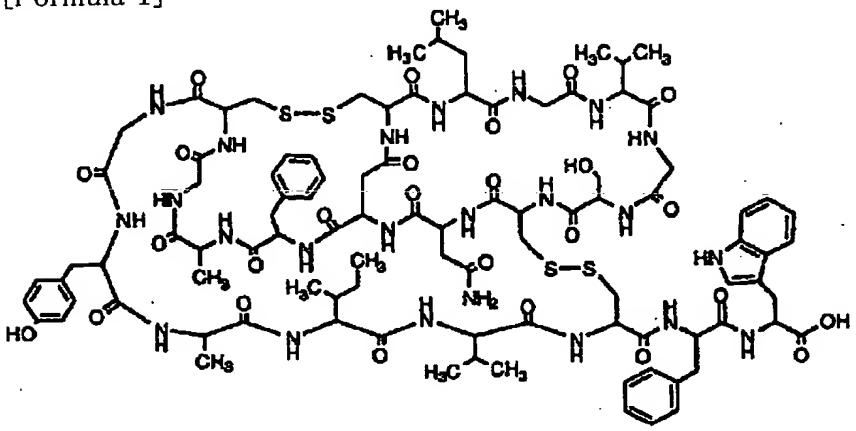
CLAIMS

[Claim(s)]
[Claim 1] The peptide compound expressed with the following-ization 1.
[Formula 1]



[Claim 2] The manufacture technique of the peptide compound which cultivates the peptide compound production bacillus belonging to a Streptomyces, and is characterized by extracting the peptide compound indicated by the claim 1 from the culture.
[Claim 3] The manufacture technique of the peptide compound whose peptide compound production bacillus belonging to a Streptomyces is Actinomyces SKH-2344 stock and with which it was indicated by the claim 2.
[Claim 4] The manufacture technique of the peptide compound indicated from the peptide compound production bacillus culture by the claim 2 or the claim 3 which uses an anion exchange resin, and extracts and refines the peptide compound indicated by the claim 1.
[Claim 5] The anti-human immunodeficiency virus agent characterized by making into an active principle the peptide compound indicated by the claim 1.

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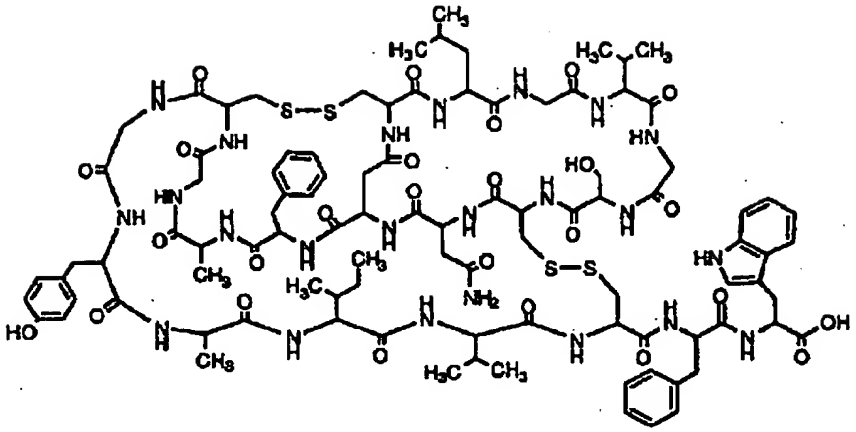
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] this invention -- a peptide compound -- being related -- especially -- anti- -- it is related with HIV (anti-human immunodeficiency virus) active-substance NP-06, the manufacture technique of those, and anti-HIV agent that contains this ** HIV active substance as an active principle
[0002]
[Description of the Prior Art] In recent years, the patient of the acquired-immunity insufficient morbus (it is called AIDS for short below) is increasing worldwide. Especially in Asia and the Africa area, it is in the status of an infection explosion of HIV which is the cause of AIDS, and it is thought that an AIDS patient will increase rapidly centering on these areas from now on.
[0003] HIV is the virus of the Retroviridae which uses RNA as a gene mainframe. Infection of HIV begins from the adsorption process specifically combined with CD4 (cluster **** day ********** ray ****** anti gene 4) protein with which the virus particle which invaded into the sanguis exists in a T cell front face through the sugar protein called gp-120 which it has on this virus-particle front face. The virus particle which stuck to the cell surface emits and carries out the cast skin of the HIV gene (RNA) into a cell, and, subsequently to DNA, HIV gene is rewritten by reverse transcriptase (reverse-transcription process). And the genetic information of HIV rewritten by DNA passes through the inclusion process incorporated by the integrase into DNA of a host cell further, and virus infection is materialized.
[0004] Within the infected cell, virus protein is produced on the basis of the genetic information of a virus. It is shown clearly that the protein which acts on the produced virus protein as a virus propagation controlling factor called Tat and Rev exists. The virus protein produced within the host cell is changed into virus configuration protein, when it is divided by the protease or a sugar chain is embellished by the ****** cytase (maturation process). Next, the viral protein and virogene which were compounded gather and a new virus particle is formed (packaging process).
[0005] The virus particle produced newly is emitted out of a cell (exudation process), and is infected with the new cell. By infection of HIV, an immunocyte usually results in a cell death by disappearing the immunity function which it originally has, or causing giant-cell formation. The patient infected with HIV lapses into the immunological deficient state of a degree very much by the decrement in an immunocyte, and the opportunistic infection by disease germ called a cytomegalovirus and the Carini protozoa is caused. Then, the medication of prescribing for the patient the medicine which checks the propagation in a cell or cell infection of a virus now is considered to bring immunity functional recovery of an infection patient or the prolongation-of-life effect.
[0006] Recently, the more detailed elucidation about the life cycle (life cycle) in the Homo-sapiens immunocyte (T cell) of HIV is being made, and the possibility as an AIDS therapeutic drug is found out by the enzyme which participates in propagation of a virus especially, and the compound which has the prevention activity of a receptor. By the way, three kinds of nucleic-acid acid system compounds called AZT (hiding place thymidine), DDC (dideoxy cytidine), and DDI (dideoxy inosine) have received license as a therapeutic drug of AIDS now. By acting on the

reverse-transcription process in the life cycle of HIV, the medicine of these nucleic-acid systems discovers anti-HIV activity.
[0007] However, when medication is performed using these nucleic-acid system compounds, the problem that anti-HIV activity decreases or disappears by occurrence of a drug-tolerance stock, and the problem that it is impossible to carry out a continuous administration for a long period of time since there is a strong side effect etc. have become clear, and there is a limitation in AIDS treatment only by the present license medicine. From such present condition, the combined use treatment using two or more medicines which have the operation mechanism from which AIDS treatment of the future is different is considered to become in use. Then, the search and development of a compound which suppress propagation of HIV specifically are newly worldwide furthered now focusing on an adsorption inhibitor, a cast-skin inhibitor, the nonnuclear acid system reverse-transcription inhibitor, the protease inhibitor, Tat inhibitor, the glycosylation inhibitor, etc.
[0008] for example, as an adsorption inhibitor Fusibility CD4 (D. others [1704 / (1987) / 238 and 1704 / H.Smith, R.A.Byrn, S.A.Marsters, T.Gregory, J.E.Groopman and D.J.Capon, Science, and]) which made CD4 the fusibility, Dextran sulfate, Sulfation polysaccharide, such as a heparin sulfuric acid (M.) [A.Skinner R.Ting, A.Langlois, KJ.Weinhold, H.KLyerly, KJavaherian and, T.J.Matthews, and] [AIDS] Others [4,187 / Res.Hum.Retorovirus and / (1988)] ;
[0009] a nonnuclear acid system reverse-transcription inhibitor ****** -- TIBO (tetrahydroimidazo-benzodiazepine-ON and - thione) (it Natures (Nature) Pauwels, R., Andries, K., Desmyter, J., and et.al. --) Others [343:470 / (1990)], HEPT (1-[(2-hydroxy ********) methyl]-6-(phenylthio) thymine) (Miyasaka, T., Tanaka, H., Baba, M., Hayakawa, H., et.al., and J.Med.Chem. (journal ****** decal chemistry) --) As 32, 2507 (1989), and a protease inhibitor Ro 31-8959 (Roberts, N.A., Martin, J.A., Kinchington, D., et.al., Science, 248,358 (1990) (science)), KNI-272 (Mimoto, T., Imai, J., Kisanuki, S., Enomoto, H., et.al., and Chem.Pharm.Bull. (chemical fur ******-tee ****** rutin) --) 40 and 2251 (1992) The grade is found out.
[0010] Moreover, it asks for the possibility of a new compound, and also comes to perform worldwide searching for anti-HIV active substance from a natural product, and anti-HIV active substances, such as RP-71955 (Gerard, H., Catherine, D., Jean-Francois, M.and Jean, L., J.Antibiotics, 46 (journal ********** biotechnology tex), 1756 (1993)), are already found. However, while excelling in anti-HIV operation, the lead compound which may serve as anti-HIV agent which is low toxicity, or anti-HIV agent is not yet found out.
[0011]
[Problem(s) to be Solved by the Invention] Then, by the screening procedure (the MTT assay method) which can measure anti-HIV activity effectively in in vitro, as a result of screening the secondary metabolite of the microorganism separated from soil, this invention person etc. found out the lead compound which is excellent in anti-HIV operation especially, and may serve as anti-HIV agent of low toxicity, and its manufacture technique, and reached this invention.
[0012] Therefore, the 1st purpose of this invention is to offer the lead compound which may serve as anti-HIV agent which is low toxicity while it is excellent in anti-HIV operation. Moreover, the 2nd purpose of this invention is to offer the manufacture technique of the lead compound which may serve as anti-HIV agent which is low toxicity while it is excellent in anti-HIV operation. Furthermore, the 3rd purpose of this invention is to offer anti-HIV agent which makes an active principle the lead compound which may serve as anti-HIV agent which is low toxicity while it is excellent in anti-HIV operation.
[0013]
[Means for Solving the Problem] Many above-mentioned purposes of this invention were attained by the peptide compound expressed with the following-ization 2, its manufacture technique, and anti-HIV agent which makes it an active principle.
[Formula 2]



[0014] this invention is explained in full detail below. this invention person etc. is in vitro for the purpose of offering anti-HIV agent excellent in anti-HIV operation. The screening procedure (the MTT assay method) which sets and can measure anti-HIV activity effectively was used. This technique is the outstanding technique which suppression of the infection death of CD4 positivity cell caused by HIV infection and the aforementioned infection death by the medicine is judged, and can measure anti-HIV activity and a cytotoxicity simultaneous and simple.
[0015] While this invention persons screened the secondary metabolite of the microorganism separated from soil using this screening procedure, they found out the matter excellent in especially anti-HIV operation, and named this NP-06. the above -- anti- -- HIV active-substance NP-06 are matter which has the following physicochemical properties while they have the structure expressed with the above-ization 2
[0016] character: -- white -- a powder molecular formula:C97H131 O26N 23 S4 molecular weight:2163 solubility:methanol, a dimethylformamide, and dimethyl sulfoxide -- ****, water, ethyl acetate, and chloroform -- refractory acidolysis product: -- a glycine (4), a cystine (2), an alanine (2), a valine (2), a phenylalanine (2), a leucine (1), an aspartic acid (2), a serine (1), an isoleucine (1), and
IR(KBr) cm-1:701, 746, 1178, 1219, 1337, 1390, 1407, 1454, 1517, 1649 (C=O), 2934, 2964, 3009, 3082 (C=C), 3306, 3317 and 3376, 3387 (OH, NH) angle-of-rotation:[alpha] D 20=-75 degree (C0.1%, MeOH)
In addition, in case of structure decision, various NMR measurement and the chemical decomposition technique were used on the basis of the data obtained from an amino acid analysis, MASS spectrum measurement, and IR measurement.
[0017] It became clear for NP-06 of this invention to have the same amino acid composition as RP-71955 (Gerard, H., Catherine, D., Jean-Francois, M., and Jean, L., J.Antibiotics, 46, 1756 (1993)) previously reported by Gerard etc., and to have the structure which was very similar also in the amino acid sequence. That is, the difference between NP-06 and RP-71955 is that the modalities of amino acid differ in two places.
[0018] this invention -- anti- -- HIV active-substance NP-06 were separated from the soil extracted from the Yamaguchi ** isthmus -- Although it is manufactured when it is isolated from the culture which cultivates the microorganism SKH-2344 stock belonging to a Streptomyces, and was obtained, therefore NP-06 of invention inoculate SKH-2344 stock into a nutrient inclusion culture medium and cultivate it aerobically What is necessary is just to have the capacity for the production bacillus of NP-06 which can be used by this invention to belong not only to the above-mentioned strain but to a Streptomyces, and to produce NP-06.
[0019] By the way, SKH-2344 stock is a microorganism which has the following mycology-properties.
1. The gestalt-characteristic feature bacteria stock was cultivated by the liquid medium which contains a yeast extract and 1% glucose 1%, and L and L-diaminopimelate was detected, when thin-layer chromatography analyzed, after hydrolyzing this biomass at 110 degrees C for 18 hours using 6N hydrochloric acid. Moreover, when microscope observation was performed about the

object made to grow on an agar-plate culture medium, the gestalt which the mind hypha is presenting the shape of a spiral, in addition should be mentioned especially did not accept.
[0020] 2. Growth Status on [Various] Culture Medium (Incubation and Color Tone Will Call at Standard Color Table of Japanese Standards Association Issue 27 Degrees C for 14 Days)
(1) yeast extractives malt extractives agar-medium growth: Good mind hypha: Many mind hypha color tones: 5RP 6 / 2 rear-face color tone: 5R 2/2 soluble pigment: nothing -- [0021] (2) oatmeal-agar-medium-Growth-:- -less (3) starch and mineral salt agar-medium growth: [ ] -- good mind hypha: [ ] -- abundant -- mind hypha Color tone: 7.5R 8/2 rear-face Color tone: 2.5GY 3/2 soluble-pigment: [ ] -- nothing -- [0022] : Good mind hypha: Abundant mind hypha color tone: 7.5R 5 / 2 rear-face color tone: 5Y 4/2 soluble pigment (4) glycerol asparagine agar-medium growth: [ ] -- good mind hypha: [ ] -- abundant -- mind hypha Color tone: 7.5R 7/2 rear-face Color tone: 2.5GY 3/2 soluble-pigment: [ ] -- nothing -- [0023] : Good mind hypha: Few mind hypha color tone: White rear-face color tone: 2.5GY 4/2 soluble pigment: Nothing (5) thyrosin agar-medium growth (6) mind hypha: [ ] with sufficient sucrose and nitrate agar-medium growth -- few mind hypha Color tone: white rear-face Color tone: 2.5GY 4/2 soluble-pigment: [ ] -- nothing -- [0024] : Good mind hypha: Nothing mind hypha color tone: - rear-face color tone: 2.5GY 6/2 soluble pigment: Nothing (7) glucose asparagine agar-medium growth: (8) nutrient-agar-medium growth [ ] -- few mind hypha Color tone: white rear-face Color tone: 2.5GY 6/2 soluble-pigment: [ ] -- nothing -- [0025] : Good mind hypha: Nothing mind hypha color tone: - rear-face color tone: 2.5Y 9/4 soluble pigment: Nothing (9) Bennett agar-medium growth: Good mind hypha : 3. Growth Temperature Requirement (for Yeast Extractives Malt Extractives Agar Medium and Seven Days)
7degree-C : [ ] -- usually -- 17 degree-C growth: -- usually -- 21 degree-C growth: -- good 25 degree-C growth: -- good 29 degree-C growth: -- good 32 degree-C growth: -- good 37 degree-C growth: -- [0026] which do usually 40 degree-C Growth: carry out, not to 45 degree-C Growth: carry out and not to 50 degree-C Growth: carry out Growth: 10 degrees C not to carry out Growth: usually -- 14 4. use D-glucose [ ] of sugar -- usually -- L-arabinose growth: [ ] -- good sucrose growth: [ ] -- good inositol growth: [ ] -- good D-mannitol growth: [ ] -- good [0027] Growth: Good D-fructose Growth: Good L-rhamnose Growth: Good raffinose Growth: usually -- D-xylose Growth: It is clear from many above-mentioned properties that SKH-2344 stock is a microorganism belonging to a Streptomyces. In addition, it is as of March 22, Heisei 6 in National Institute of Bioscience and Human-Technology, the Agency of Industrial Science and Technology, and a bacteria stock is FERM. It ****s as P-14239. In principle, although the incubation technique of the above-mentioned microorganism applies to the incubation technique of a common microorganism, it is suitable for it to usually carry out by the shaking cultivation by the liquid medium or the aeration spin-culturing method. Any culture media, such as various kinds of synthetic mediums, a semisynthetic medium, and a natural medium, can be used that what is necessary is just a culture medium containing the nutrient which can use the microorganism belonging to a Streptomyces as a culture medium used for incubation.
[0028] independent [in a glucose, shoe cloth, a fructose, a glycerol, a dextrin, molasses, a cone and a ********** car, an organic acid, etc.] as a carbon source of a medium composition -- or it can be combined and used independent [in inorganic nitrogen sources, such as organic nitrogen sources, such as a peptone, a meat extract, a yeast extract, a soybean meal, casein, a urea, and amino acid, an ammonium sulfate, and a sodium nitrate,] as a nitrogen source -- or it can be combined and used The heavy metal etc. and the vitamins of a specific salt, potassium salt, magnesium salt, phosphate, and others can also be used if needed. In addition, during incubation, when a foaming is remarkable, well-known defoaming agents, such as a ****** Norian and a silicone oil, can also be suitably added in a culture medium.
[0029] As for pH of a culture medium, it is desirable to consider as near [which a microorganism grows good] neutral. Moreover, as for the temperature of a culture medium, it is desirable to consider as 10-37 degrees C which a microorganism grows good, and it is desirable to keep at 25-30 degrees C especially. Furthermore, generally incubation time will be about three - six days. These culture conditions can be suitably changed according to the conditions to use, such

as a modality of microorganism, and a property. NP-06 are accumulated in a biomass and a culture medium by the above-mentioned incubation.
[0030] the isolation of NP-06 produced by the above-mentioned incubation is independent according to the general technique of extracting a production-by-fermentation object, in various meanses, such as a salting-out method, an extraction method, various gel chromatographies, an ion exchange chromatography, and an adsorption chromatography, when a store becomes the maximum preferably, after accumulating NP-06 in a culture (a biomass and culture medium) -- or it combines and carries out In addition, NP-06 are detectable by the bioassay method, the HPLC method, the TLC method, etc.
[0031] That is, since NP-06 produced by the above-mentioned incubation exist in both the inside of a biomass, and in a culture medium, after they separate incubation filtrate and a biomass solid content with meanses, such as filtration and centrifugal separation, they extract NP-06 using solvents, such as a methanol and an acetone, carry out the organic solvent reduced pressure and **** by the evaporator etc. further if needed, and obtain extraction concentration liquid from a biomass solid content.
[0032] The specified substance is efficiently recoverable out of an extract and incubation filtrate by making Amberlite IRA-402, Amberlite IRA-904, Amberlite IRA-958, Amberlite IRA-68, Amberlite IRA-93, and Amberlite IRA-35 (tradename of the anion exchange resin by ORGANO CORP.) adsorb and elute. Moreover, n-butanol, ethyl acetate, etc. may extract and recover the specified substance.
[0033] Subsequently, after condensing the extract containing the specified substance, the chromatography using diamond ion HP-20 column, sephadex LH-20 column, the silica gel column, the antiphase column, etc. refines. Thus, a crystalline cellulose etc. can be added to NP-06 obtained and extremely excellent anti-HIV agent which makes NP-06 an active principle can be obtained by considering as a tablet if needed, so that it may be well-known.
[0034]
[Effect of the Invention] Since toxicity is low while excelling in anti-HIV operation, as for taking for a long period of time, NP-06 which are the peptide compound of this invention are also possible in large quantities, and are very promising as a new AIDS therapeutic drug.
[0035]
[Example] Hereafter, this invention is not limited by this although this invention is further explained in full detail according to an example. In addition, % means weight %, unless it is specified as others.
[0036] centrifugal separation separated into the biomass and the culture supernatant, after having come out of Actinomyces SKH-2344 stock and cultivating for five days at 25 degrees C using the jar fermenter of 1.30l. of examples by the 20l. culture medium (glucose 20g, starch 10g, 1g of malt extracts, 4g of dry yeast, 25g of soybean meals, 2g of salt, 54mg of potassium phosphate, 1l. of water, pH 7.3)
[0037] In the 10l. methanol, the biomass removed the residue by filtration overnight, after carrying out a bacteriolysis, stirring and. After making a methanol solution harden by drying with vacuum concentration, the obtained solid content was melted in the water-saturation butanol, equivalent water washed, and active ingredients were collected in the butanol layer. On the other hand, after flooding with 1l. of methanols the hardening-by-drying object which carries out vacuum concentration of the culture supernatant, and was obtained overnight, filtration removed insoluble matter. Subsequently, after hardening a methanol solution by drying with vacuum concentration, the obtained solid content was melted in the water-saturation butanol, equivalent water washed, and active ingredients were collected in the butanol layer.
[0038] After having set two butanol solutions containing an active ingredient and ****ing a butanol, the obtained active ingredient was melted in the little methanol, and the silica gel column chromatography was given (the 4cm x length of 25cm of the diameters of a column). As an expansion solvent, the mixed solvent of ethyl acetate, a methanol, and water (10:5:0. 25) was used. The active ingredient was eluted when the expansion solvent of the amount of about 2 times of a column volume was passed. Vacuum concentration of the activity fraction was collected and carried out, and the high performance chromatography (the 2cm x length of 30cm of the diameters

of the ODS-AQ column made from column;YMC) was given. As a move solvent, 38% aqueous solution of 0.5% of a trifluoroacetic-acid inclusion acetonitrile was used. Activity fractions were collected, the solvent was ****ed and white powder was obtained. NP-06 obtained from 20l. culture medium were about 100mg.

[0039] Next, following analysis and various following activity examinations were performed about NP-06 of the obtained white powder.

1. As amino-acid-analysis following of NP-06, the configuration amino acid analysis of NP-06 was performed with the hydrochloric-acid adding-water part solution method. The configuration amino acid analysis of NP-06 was performed with the hydrochloric-acid adding-water part solution method. The reduced pressure sealed tube of the 200micro l of 6 hydrochloric acids of N was added and carried out to the test tube for amino acid decomposition into which 0.2mg of the reduced-pressure-drying powder of NP-06 was put. At 110 degrees C, 24 hours and after hydrolyzing, respectively, the reduced pressure drying of the sample was carried out on NaOH for 72 hours.

[0040] After melting the obtained solid content in 50micro l of water, the amino acid analysis was performed using amino-acid-analysis equipment. An amino-acid-analysis result (actual measurement) is as having been shown in Table 1.

[Table 1]

	Mol比	
アミノ酸	24時間	72時間
Asp	1.93	2.18
Ser	0.86	1.14
Gly	3.55	4.27
Ala	1.90	2.38
Cys	2.23	2.28
Val	1.47	2.01
Ile	0.52	0.84
Leu	1	1
Tyr	1.07	0.86

In addition, since a tryptophan decomposes into an acidolysis reaction, by the above-mentioned technique, it is not detected, but it is decomposed and an asparagine becomes an aspartic acid.

[0041] 2. The C terminus amino acid analysis of NP-06 was performed by the C terminus amino-acid-analysis hydrazinolysis method of NP-06. First, the reduced pressure sealed tube of the 100micro l of anhydrous hydrazines was added and carried out to the test tube for amino acid decomposition into which 2mg of the reduced-pressure-drying powder of NP-06 was put. After performing a decomposition reaction at 100 degrees C for 18 hours, it dipped in 2ml DEAE resin column (tradename of the column by TOSOH CORP.), the hydrazide of a by product was removed, and C terminus amino acid was refined.

[0042] The obtained C terminus amino acid was identified by TLC [silica gel 60Fmade from TLC plate:Merk: [245 and expansion solvent] (1) chloroform / a methanol / 28% aqueous ammonia (2:2:1) (2) n-butanol / acetic acid / water (3:1:1), and a detection:ninhydrin reagent], and that they are a result and a tryptophan made it clear.

[0043] 3. NMR Structural-Analysis Use Device:JNM of NP-06 EX-400 (Tradename made from JEOL)

Measurement Condition: 1 measurement solvent:DMSO-d6 (internal-standard TMS), sample concentration:100mM. Measurement Temperature: 25 degree-C2 measurement solvent:CD3 OH-H2 O (50%-50%) (internal-standard TMS), sample concentration:10mM. Measurement Temperature: 40 degree-C measurement data:HH-COSY spectrum (DQF-COSY spectrum) HOHAHA spectrum NOESY spectrum [0044] (1) A leucine (Leu), an isoleucine (ILe), valine (Val) x2, alanine (Ala) x2, glycine (Gly) x4, and 11 amino acid of a serine (Ser) were checked among 21

amino acid in the analysis of HH-COSY, HOHAHA spectrum-analysis result HH-COSY, and HOHAHA spectrum. The ten remaining amino acid residues showed the same AMX spin sequence, and have not distinguished it by HH-COSY and HOHAHA spectrum.

[0045] (2) By the NOESY spectrum-analysis result HOHAHA spectrum, identification about ten amino residues which cannot be identified was performed. About each amino acid residue of phenylalanine (Phe) x2, a thyrosin (Tyr), and a tryptophan (Trp), it identified according to NOE correlation peak of a phenyl group and beta-proton, respectively. About the remaining 6 amino acid residues, it identified with the chemical shift value of cysteine (Cys) x4, asparagine (Asn), aspartic-acid (Asp), and Hbeta of an amino-acid-analysis result.

[0046] The chemical shift value (ppm) of the identified amino acid is shown in Table 2.

[Table 2]

アミノ酸	NH	Hα	Hβ	Hγ	Hδ
Cys(1)	8.7	4.7	3.5、2.5		
Leu(2)	9.8	4.5	1.65、2.1	2.05	1.1(CH_3)、0.97(CH_3)
Gly(3)	8.65	4.1、3.7			
Val(4)	7.1	4.17	1.87	0.83(CH_3)、0.85(CH_3)	
Gly(5)	7.1	3.65、4.45			
Ser(6)	8.0	4.5	4.0、3.8		
Cys(7)	8.15	5.0	3.5、3.1		
Asn(8)	8.85	5.2	2.5、2.4		
Asp(9)	8.05	4.5	2.8、2.9		
Phe(10)	8.6	4.35	2.9、2.7		
Ala(11)	8.5	3.95	1.3(CH_3)		
Gly(12)	8.85	4.2、3.8			
Cys(13)	8.3	4.4	3.2、3.4		
Gly(14)	7.45	3.3、3.9			
Tyr(15)	9.4	5.5	2.1、2.75		
Ala(16)	8.4	4.7	1.3(CH_3)		
Ile (17)	8.17	4.3	1.83	1.15、1.55、0.95(CH_3)	0.9(CH_3)
Val(18)	7.35	3.9	1.87	0.75(CH_3)、0.87(CH_3)	
Cys(19)	7.8	4.45	2.15、2.2		
Phe(20)	8.15	4.54	2.6、2.9		
Trp(21)	7.4	4.55	3.25、3.15		

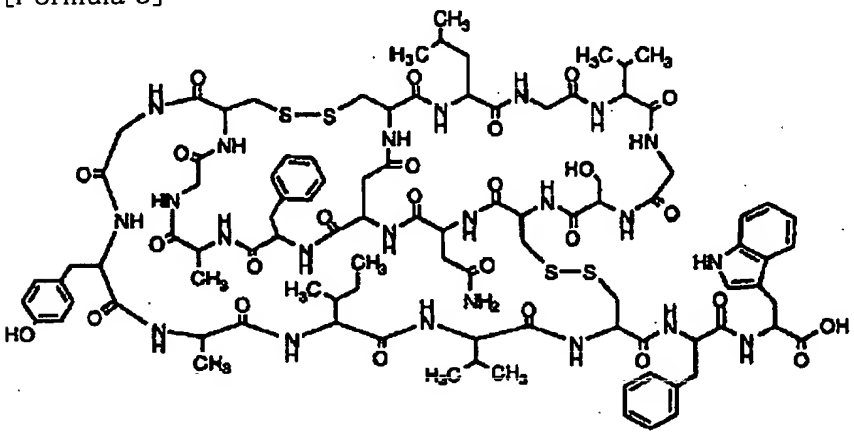
[0047] (3) It is the array of 21 amino acid residues identified by amino-acid-sequence NMR of NP-06 Halphai-HNi+1 It determined by reading by NOE intensity of a between. From the amino acid of a C terminus, since only the correlation peak with Halpha of HN and Phe (20) of Trp (21) accepted, Trp (21) was determined as C terminus amino acid. This result is in agreement with the result of the C terminus amino acid analysis by the hydrazinolysis method. NOE on-the-strength analysis result is shown in drawing 1 , and the amino acid sequence of NP-06 is shown in drawing 2 .

[0048] (4) whether it combines and comes out and SS combination is formed and what ** [be / important / in the case of the primary structure analysis of primary structure analysis NP-06 of

NP-06 / it / Cys / **4 **] -- the amino terminus amino group of Cys (1) is whether to be in what status Then, the Longe Range NOE correlation peak was examined in detail about these two points. From the correlation peak with Hbeta of HN and Asp (9) of Cys (1), it checked that the amino terminus of Cys (1) and beta grade carboxyl group of Asp (9) were carrying out amide combination. According to the correlation peak of Hbeta of Cys (1), and Halpha of Gly (14), it checked that Cys (1) and Cys (13) were carrying out SS combination. According to the correlation peak of Hbeta of Cys (7), and Hbeta of Cys (19), it checked that Cys (7) and Cys (19) were carrying out SS combination. An analysis result is shown in drawing 3 .

[0049] The primary structure of NP-06 was determined as ** 3 on the basis of the above result.

[Formula 3]



Although this structure is very similar with RP-71955 reported recently as it was mentioned above, the positions of Val (4) and ILe (17) differ.

[0050] 4. NP-06 -- anti- -- the anti-HIV activity of HIV activity NP-06 was measured by the test method of the following which used MT-4 cell This technique is the outstanding technique which the suppression by the medicine is judged to be the infection death of CD4 positivity cell caused by HIV infection, and can measure anti-HIV activity and a cytotoxicity simultaneous and simple.

[0051] (Test method) 1x104 MT-4 cell infected with HIV-1 (HTLV-3B) of an individual and MT-4 non-infected cell were added to each hole of 96 hole microplate with NP-06 of various concentration. 37-degree C CO2 3-(4, 5-dimethyl-2-thiazolyl)-2 after cultivating for five days in an incubator, and 5-diphenyl-2H tetrazolium bromide (MTT) -- adding -- further -- incubation was continued for 2 hours It is returned by the enzyme which the mitochondrion in a cell has, and MTT incorporated by the viable cell in the meantime is water-insoluble nature coloring matter (formazan) of a bluish violet color. It generated. The hydrochloric-acid acidity 2-propyl alcohol solution containing 5%Triton X-100 was added, and after making the generated coloring matter solubilize, the difference of both absorbances was searched for by measuring the specific absorbance in 595nm, and the un-specific absorbance in 655nm using a microplate reader (BIO-RAD model 3550).

[0052] Computer processing (BIO-RAD Microplate Manager for Macintosh) was performed using the acquired numeric value, it asked for cell-death prevention concentration (EC50) and 50% cytotoxicity concentration (CC50) 50%, and the therapeutic index (CC50/EC50) was computed. NP-06 and anti-HIV assay result of dextran sulfate natrium (molecular weight 5000) (comparison medicine) already reported for the anti-HIV effect to be clear are shown in Table 3.

[Table 3]

測定項目 / 薬剤名	EC50(μg/ml)	CC50(μg/ml)	治療係数(EC50/CC50)
NP-06	1.35	66.45	49
デキストラン硫酸ナトリウム (分子量5000)	0.45	>100	>220

[0053] 5. If the giant-cell formation prevention effect HIV of NP-06 is infected with a T cell, it is known that an infected cell will unite mutually and will form a giant cell. Then, the giant-cell formation prevention effect of NP-06 was examined.
(Test method) 5x104 The HIV-1non-infected MOLT cell of an individual, and HIV-1 persistent-infection MOLT-4 cell with NP-06 of various concentration It adds to each hole of 96 hole microplate, and is 37-degree C CO2. After cultivating in an incubator for 24 hours, observation or photography was performed using the microscope, the grade of multinucleated-giant-cell form depressant action was observed, and anti-HIV-1 activity (HIV-1 adsorption prevention activity) was evaluated.
[0054] It is as having been shown in Table 4, NP-06 concentration is 10micro more thang/ml, and the giant-cell prevention effect was checked by the result.
[Table 4]

薬剤濃度 (μg/ml)	50	10	2	0.4
巨細胞形成阻害判定	巨細胞形成阻害有	巨細胞形成阻害有	巨細胞形成阻害無	巨細胞形成阻害無

[0055] Like the example 2. example 1, centrifugal separation separated into the biomass and the culture supernatant, after cultivating Actinomyces SKH-2344 stock for five days using a 30l. jar fermenter by the 20l. culture medium (glucose 20g, starch 10g, 1g of malt extracts, 4g of dry yeast, 25g of soybean meals, 2g of salt, 54mg of potassium phosphate, 1l. of water, pH 7.3). After stirring a biomass in a 10l. methanol overnight, filtration removed the solid content. Subsequently, vacuum concentration was carried out, the methanol was ****ed and the aqueous solution was obtained.
[0056] After preparing pH to 7.0 together with a culture supernatant, it dipped in the anion exchange resin (an Amberlite IRA-93 (tradename by ORGANO CORP.) hydrochloride type, diameter of column 8cmx50cm), and the active ingredient was made to adsorb. 0. After each column carried out amount dipping of 5M monopotassium-phosphate liquid (pH4.5), water, and the 50% methanol 3 times at order, the active ingredient was made eluted with a methanol solution 50% of 0.1M acetic-acid inclusions. Reduced pressure hardening by drying of the activity fraction was carried out, and it obtained 320mg of rough refining objects (about 80% of purity). The effectiveness of this invention is proved [result / above].

[Translation done.]